USDA-ARS Cacao Research Meeting

Miami, FL

January 20-24, 2002

MEETING SUMMARY

The USDA/MARS sponsored meeting on Cacao Research was held in Miami from January 20-24, 2002. Participants at the meeting were from 10 countries representing 14 research institutes and two commercial companies. The focus of the meeting was collaboration on research to develop disease resistant cacao cultivars. The first day was devoted to research reports for each institution. The second and third days were devoted to four areas of discussion, 1) EST, Microarray, BACs and Gene discovery, 2) breeding populations, 3) pathology, and 4) administrative coordination; the reports from each of these discussion groups are included. The last day was devoted to an INGENIC proposal for collaboration to produce a 'International Working Group on Cocoa Genome Studies'. The working group was established and the details of the INGENIC meeting are listed in the report. It was agreed by the group that another meeting should be held in February or March 2003 in a cocoa producing country.

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USDA ARS Cacao Research Meeting
Miami Florida Jan. 20-24 2002

Participants' Ideas: Opportunity for Greater Individual/Institutional Collaboration

- As one strategy to attract increased donor/sponsor attention for cacao research and funding, we should find ways to link our topic (cacao) to other global issues of concern which already have recognized wide support. For example, issues of economics, poverty, support for agriculture in developing countries, global warming, the environment, reforesting, democracy, etc.

- We need to better identify where and how our various institutional research programs can connect for greater synergy and efficiency. We also must identify any research gaps that now exist, and plan to address these. Some would say there is currently a degree of "chaos" across our disparate scientific efforts. As we "get our own house(s) in order", we will be collectively recognized as more of a coordinated community, a critical mass of capability, a number of scientific experts enjoying a workable structure…rather than separate small research efforts. Scale attracts donors.

- Molecular biology of cacao is one science area in need of an increased effort.

- Many additional funding possibilities, both regional and international, may be open to fund research undertakings: work to get on government agendas for providing foreign assistance…seek grants from established global bodies such as the World Bank…document our progress and ability to deliver results and publicize/feedback to current and potential donors…extrapolate what further achievement might be possible in the future if donor support is increased.

- Additionally, the cacao challenge must not be seen as "an industry problem", with various parties sitting back and waiting for big business to solve things.

- The farmer…we can't forget this critically important end-user. Farmer focus is a key commonality among our various science initiatives. We must ensure that our science actually reached the farmer, they are engaged by our field personnel, and changes occur for the better… e.g. as in stabilizing their income level. A key challenge for us lies within the Southern Hemisphere.

- Timing can be important. For example, there is a possible danger in our taking scientific advances and new techniques too early to the farmer, especially where they have not been involved earlier as participants and input-givers. We must also promote/deliver "complete systems and answers" to the farmer, well-integrated solutions. And, as a postscript, let's not forget that farmer motivation is affected by cacao price levels…e.g. when world supply is high, prices may be low, negatively impacting farmers and others to participate in new higher-yielding/preventative agriculture techniques.

- Finally, as researchers, we might learn by looking at other commodity models including sugar, tea, etc. Although different, there may be pieces of wisdom that can be used.

Additional Comments (Steve Brown)

Transfer of end results to users, be they breeders, farmers, or whoever, is critical. To identify and/or develop probes linked to disease resistance is all well and good. However, if the breeders in producing countries do not have the resources with which to use them, then how much good do they do? Screening future progeny with markers is not insignificant in cost. If the process is too expensive for breeders in producing countries, then that defeats the purpose of developing the markers in the first place.

Maybe at this point, we need to include an "extension" phase in our program to make sure that as much research as possible is actually used. In Cote D'Ivoire, farmers will not use resistant clones or even buy seed from elite crosses if they have to pay for the seed, as they are too poor (so Dr. Kebe told us). Instead of using the hybrid seed, they will go to a neighbor and get seed from him. This seems to be an angle which must obviously be addressed if the fruits of this research are to be applied.

ESTs, Microarrays, BACs and Gene Discovery Group

January 22, 2002

Attending the session were:
•USDA, Miami (David Kuhn [FIU], Marty Heath, James Borrone, Wilber Quintanilla, Cheryl Krol)
•USDA, Beltsville (Bryan Bailey)
•Mars (David Allaway, Andrea Cataruzza, Paul Jones, Alan Bennett [consultant])
•CIRAD (Claire Lanaud)
•UESC, Brazil (Julio Cascardo)
•Penn State University (Mark Guiltinan)

Resources - Currently Available or Possibly Available (?)

•Automated sequencing and fragment analysis [all groups] This did not appear to be the limiting factor with regard to producing EST libraries. Everyone was concerned with the cost of sequencing.
•Web site for sharing of sequence information [J. Cascardo] Dr. Cascardo reported the next day at the INGENIC meeting that UNICAMP had agreed in principle to set up a website for the efficient sharing of sequence information for the EST group.
•BAC library of SCA6 [CIRAD] The BAC library seems to be at least 6 months from a form which the CIRAD group felt would be useful for sharing or availability.
•A 1200 member cacao EST library will be made available in the form of microarray slides of oligonucleotides from the Nottingham group [Paul Jones, Mars]. Potential availability is at least three months as the oligonucleotide array must first be tested.

Action Points

•Collaborate to produce as large and comprehensive an EST library as possible (all groups)
•Create a website to share EST data (J. Cascardo) [This has already been tentatively arranged]
•Provide EST library production training (Mars) [Depends on individual producers choice of library construction. Mars has produced their library in LambdaZap.]
•Release of cacao EST library sequence information (not clones) [This has already been tentatively arranged]

EST Libraries

Participants "agreed" to produce EST libraries from the following tissues. Method of library construction and initial screening of libraries has still to be discussed. Drs. Lanaud, Bailey and Kuhn agreed that library construction could begin without need for initial funding. Other participants will be contacted with regard to need for funding for library construction.
•Leaf, bean [Jones, Mars] This library should be available as an oligonucleotide microarray (see above).
•Flower [Guiltinan, Penn State] May be included as part of an NSF Floral Development program.

•Induced/Uninduced tissue culture [Bailey,USDA-Beltsville]
•Pollen [Cascardo, UESC]
•Disease challenged [Kuhn, USDA-Miami]
•Developing seed [Lanaud, CIRAD]
•Herbicide treated [Bailey, USDA-Beltsville]

Collaboration Agreements

•Each group will produce libraries using the same protocols and vectors to make eventual sharing of clones possible. [This agreement needs further discussion by participants, as not all groups want to use LambdaZap for library construction. In addition, the question of genotypes used for libraries needs further discussion.]
•Common goal is to generate sequences (at least 2,000 from each library) and share sequence data through a website. [Further discussion is required by participants with regard to screening out most common sequences from the libraries. Extensive sequencing of libraries will require additional funding but initial library construction and characterization will be used as the basis to develop a proposal.]
•Each group will initially print microarrays of their libraries for local use.

Bumps in the Road

•Determine a common philosophy regarding sharing of information. [This was dealt with by the formation of a committee headed by Mark Guiltinan, Penn State, to draft an intellectual property agreement that would satisfy the agencies of all participants.
•Identify scale of collaborative project and funding sources appropriate to the scale (ACRI & CAOBISCO for small scale project, NSF for large scale) [Development of a proposal will be decided after some library construction and collaboration has occurred.]

Report from breakout group on breeding applications of molecular markers.

Steve Brown was designated as chair and used a list of the molecular marker projects either underway in Miami or planned for the coming year as a starting point for the discussion. The topics listed on the agenda were covered within the context of the discussion. The topic of population development is a bit premature for this group, as it depends on the data that is being analyzed from the crosses already existing in the different countries. Most action points relevant to this group are contained within the cooperative agreements developed for each individual group, and many were discussed during the meeting. Any other action points will be noted in the minutes as they arise, and will be printed in **bold** type. Lizz Johnson served as the recorder.

List of cooperative projects with Miami and Central and South American Teams:

•Brazil- SCA6 x ICS-1-(F2 population) 150 trees.
•Trinidad-SCA6 x ICS-1 approx.167 trees.
It will be of great interest to compare these two analyses, and if phenotypic data is sufficiently compatible, a joint analysis will be performed as well.

•Puerto Rico- 5 Families, Bulk Seg. Analysis:
UF668xPound7, IMC67xUF613, **EET400 x SCA12, SCA6 x EET62, INC67 x SCA12**
(Populations with one parent containing Witches' Broom Resistance in bold.)
Plans exist to intercross high-yielding progeny which have been cloned and tested for 3 years in replicated trials. This gives us the ability to map genes for disease resistance and high yield by selfing and intercrossing these progeny in proven tree mapping designs (Line-origin probabilities for QTL detection-C. A. William, PAG X, 2002).

•Costa Rica- Catongo x Pound 12- 140 trees:
UF273 x P7- 260 trees:
•Ecuador- 3 populations, ca. 200/population:
.
•Genotyping of farmer selections: ~200 trees x 50 primer sets

•Internal reference database of primary clones, approx. 50 – 100 clones, using all possible primers.

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1. General aspects of markers, their applications, software, and experiences were discussed. It was pointed out by Steve Brown and generally appreciated that the quality of phenotypic data needed to be higher than that for normal field selection. Since we have an F1 population of SCA6 x ICS-1 in Trinidad and an F2 of the

same population in Brazil, a great deal of time was spent discussing how to harmonize the data going into the response vector (Y vector) for mapping. The advantages of mapping both populations individually and simultaneously was pointed out and appreciated. Lizz Johnson and Uilson Lopes spent a great deal of time discussing methodology for measuring phenotypic response for *Crinipellis*. Juan Carlos Motamayor emphasized that the development of the brooms must be scored in addition to the number of brooms. Lizz pointed out that she also had a bounty year for measuring black pod response, and Uilson will take similar data in Brazil. **All details were not worked out at the time, but will be done via internet.** A great deal of experience was shared about measuring the two diseases. **Juan Carlos Motamayor offered to write up a protocol which will be distributed for comment and modification by participants.**

2. JoinMap from Plant Breeding International in Wageningen, The Netherlands, is the only software (which anyone in the group knew about) that will **correctly** handle mapping in an F1 population from two heterozygous parents. The software is rather expensive (see prices on website: www.joinmap.nl), but does a good job, has many nice options, and has the option of making combined maps over different populations, even when the populations are of different types (e.g. F1 from heterozygous parents vs. F2 from a selfed clone of one cross of the F1). Its counterpart, MapQTL, will also do QTL mapping in F1 populations from heterozygous parents, and is the only software which was known that handles the type of populations that we commonly encounter in cacao. This program has the option of doing conventional interval mapping, cofactor mapping, and non-parametric mapping. Some people have used QTLCartographer, but Steve Brown called the author, Zhao Bang Zeng, at North Carolina State University, and Zhao Bang said quite clearly that it is NOT currently capable of handling populations like we commonly have in cacao.

3. It was decided to compose a list of approximately 150 clones most widely used and generally considered to be of high importance and to ask Jim Saunders' lab to produce fingerprints of these. The same clones may also be brought in from Africa and Asia. The idea is that basic cocoa breeding comes back to very few clones, and it would be of great value to know how close these basic clones are in the various nurseries around the world, and how many mistaken identities might be floating around in general. This will allow the use of existing crosses to continue further breeding. **Action points: Rob Lockwood, Lizz Johnson, Uilson Lopes, and Juan Carlos Motamayor are to begin to compile lists of important clones. These can be emailed to Steve Brown who will compile a joint list, eliminate duplicates, etc. At some point it will be sent out for proofing, then finalized.** The idea was brought up to construct a database with field data from crosses using the most common clones internationally in the ICGD database. The information concerning the molecular identification of the parents of the crosses must be included in this database. The idea is to take advantage of already existing crosses to continue further breeding.

4. Some discussion on non-nuclear effects on *Crinnipellis* response ensued. Uilson Lopes stated that these effects had been seen. Juan Carlos Motamayor explained that mitochondrial genome inheritance is different in some populations.

5. Steve Brown began a discussion concerning the two experiments which had been grown in Puerto Rico, from which 40 individual tree selections were made, cloned, and grown out over 3 locations. Since 3 of the crosses contain SCA clones which can be donors of genes for *Crinnipellis* resistance, and since some of the trees contain the genes for high yield which are known to exist, a breeding design can be set up with these crosses with a design used commonly in forestry breeding. This design will allow seed replication of the generation to be grown out, and will allow the experiment to be grown simultaneously in countries in which *Crinnipellis* and other diseases exist. Details of this will be sent out. (This design was discussed in general terms at the recent PAG Meetings in San Diego by Claire Williams.) We are quite lucky that these crosses were planted and that careful data was taken for the early generations.

   Rob Lockwood mentioned previous research that showed how little power single tree selection can have. This could add to the explanation as to why the trees which initially yielded so much higher than average lost a great deal of their yield advantage when cloned and grown in replicated trials.

   In spite of the loss of the yield superiority of the selections made in Puerto Rico, it is felt that this material will allow a design to be constructed that will provide seed for a multi-country experiment with disease selection possible in some selection locations.

6. Plans for the crosses in Costa Rica were discussed (Catongo x Pound7, UF273xP7) and Wilbert Phillips gave updates on how much data had been collected, etc. Plans were discussed for mapping, etc. Juan Carlos mentioned that Nestle was willing to share data for the backcross on which RAPD's and AFLP had already been done. Wilbert Phillips mentioned that even though the backcross was susceptible to Monilia, some resistance was seen (seemed recessive, similar to what Steve Tanksley has widely reported).

7. Juan Carlos Motamayor returned recently from Ecuador where 6 populations are available for mapping with good numbers in each. Miami plans to go forward with 3 populations, hopefully this year. **Steve Brown will be in touch with Carmen Suarez about getting the phenotypic data already collected. Steve and Juan Carlos will visit Ecuador probably in early April.**

8. VB's (farmer selections) were brought up and are felt to be valuable. It will be interesting to see if they have only known genes from SCA sources or other sources as well. Leaves from several selections were brought to Miami from Trinidad by Lizz Johnson. Several have been sampled also from Brazil. When

sufficient markers are obtained in Miami to sample the genome thoroughly, it will be interesting to see which known clones these are most related to. Hopefully we will learn of new sources of witches' broom resistance other than the SCA's.

9. Steve Brown mentioned that Miami wants to construct a basic clonal database of 50-100 of the most valuable clones and saturate the database with all SSR markers possible. This database will be useful for many things: classifying incoming germplasm, choosing crosses for QTL mapping experiments with sufficient polymorphism, etc. With well saturated genomes, this database could, with the use of association genetic techniques, suggest regions of interest for fine-mapping, sequencing, SNP detection, etc. As this is basically the same as the previously mentioned project (#3), perhaps it would be better for the molecular data to be done in Miami and to use the list developed as discussed in point #3.

10. Bertus Eskes mentioned that clones to be crossed for a multiple location QTL analysis should be selected in a few months for the second phase of the CFC project. Rob Lockwood and Ray Schnell emphasized choosing clones from breeder's perspective. (A subsequent discussion was held on Thursday led by Bertus Eskes placing these ideas into a more formal context. A large project was planned which will be grown out over many countries to attempt to map differential QTL in different locations and to measure QTL x location interaction where it exists.)

11. Discussion was held about what kind of end product we might want from breeding projects: clones, F1 hybrid seed, or seed in other genetic state. Steve Brown brought up the subject of producing haploids from anthers, for dihaploid production with subsequent F1 seed production. Mark Guiltinan stated that past results did not look terribly optimistic for dihaploid production. Juan Carlos Motamayor suggested that molecular analysis could allow the selection of homozygous parents as testers for producing hybrids.

12. The idea was brought up to include in the ICGD a catalog of crosses made with worldwide data from evaluation trials. This could be a very valuable reference for breeders. (Could this be funded by CFC (Bertus) and USDA???

13. The idea was brought up that Ecuador might be a good location in which a major cocoa improvement effort could be funded and established: there is tremendous variability of germplasm, all major diseases are present in the natural environment, there is room for extensive yield trials, etc.

Pathology working group

22 January 2002

Those in attendance for all or part of the session were:
Ken Gillespie (MARS)
Karina Gramacho (CEPLAC)
Prakash Hebbar (MARS)
Ulrike Krauss (CATIE)
Smilja Lambert (MARS)
Bob Lumsden (ACRI)
Wilbert Phillips (CATIE)
Hank Purdy (UF)
Bob Schmidt (UF)
Ray Schnell (USDA-ARS)
Carmen Suarez (INIAP)
Cecile Olano, recorder (USDA-ARS)
Randy Ploetz, chair (UF)

Diseases are one of the main constraints to cacao production worldwide, and are a principal reason for convening this meeting. During this session, three areas were considered: 1) the major diseases, 2) the major research objectives and data gaps, and 3) action points for the major objectives.

The diseases of cacao that were considered were:

1. **Black pod** (caused by *Phytophthora capsici, P. citrophthora, P. megakarya and P. palmivora*). This disease is most destructive due to its global distribution and serious impact on pod yield and quality.
2. **Witches' broom** (caused by *Crinipellis perniciosa*). This is the worst problem in the Americas, and poses the greatest potential for damage if the pathogen were to be introduced outside its present range (e.g. north of Panama, and Africa and southern Asia).
3. **Frosty pod** (caused by *Moniliophthora roreri*). This disease has the most restricted distribution of the "big three" diseases, and is a serious threat to areas outside its present range (e.g., Brazil, Africa and southern Asia).
4. ***Ceratocystis fimbriata***. This pathogen has a wide host range, but has been reported as a problem on cacao only in certain areas in the Americas (e.g., Brazil and Ecuador). It is soilborne, but is effectively vectored by a beetle (*Xyleborus* sp.). Work from CEPLAC indicates that new cacao hybrids that resist witches' broom may be more susceptible to the fungus. The lethal disease the pathogen causes warrants monitoring as an emerging threat.
5. **Swollen shoot** (caused by *Cacao swollen shoot virus*). The pathogen is a variable pararetrovirus (badnavirus) that is vectored by mealybugs. Swollen shoot is a new encounter disease in Asia and West Africa. The pathogen poses a risk if germplasm were to be moved out of the affected countries.

6. **Vascular streak** (caused by *Oncobasidium theobromae*). This is a relatively new disease in Asia (the original host is not known). The pathogen is a nonculturable obligate basidiomycete. It is now widely spread in southern Asia and poses a serious risk if germplasm were to be moved out of the affected countries.
7. **Rosellinia root rot** (caused by *Rosellinia* sp.). Members of this genus usually have wide host ranges. Rosellinia root rot of cacao is a relatively minor problem in Latin America. The disease can interact synergistically with that caused by *C. fimbriata*, and in the latter case losses of up to 7% have been reported.

CURRENT STATUS AND OBJECTIVES FOR FUTURE WORK

The current status of work in several different areas is outlined below. Action points for the most important of these are listed at the end of the report. Work in the latter areas should be supported when grant funds are available.

Standardization and improvement of early screening techniques
Black pod. There is a wide consensus that the existing leaf disk assay for black pod resistance is convenient and provides reproducible results. Wider recognition is needed of the differential impacts that different pathogen species have on different cacao clones.
Witches' broom. The conveyor belt method that was developed at the University of Florida, currently utilized by CEPLAC-Itabuna and INIAP-Pichilingue, is effective and should be used more widely. For it to be effective, several factors must be recognized and well controlled: it should be noted that it took considerable time before those at CEPLAC identified and understood factors that affected results under their conditions. The important factors include: continuous free moisture on host surfaces for 24 hours after inoculation; temperature ideally between 24^{o} and 26^{o}C, and never above 30^{o}C or below 15^{o}C; physiological state of the host (inoculated tissues should be new vegetative flushes that contain at least one leaf of ca. 0.5 cm in length); and high quality inoculum. Failures of the system in other locations have likely been due to a lack of attention to these details.
Frosty pod. There is a critical need to develop a reliable technique for rapidly identifying resistance to this disease. The seedling assay of Harry Evans (1978) should be refined and examined for reproducibility. This is a possible future focus of Wilbert Phillips (CATIE).
Sap test. A sap test for witches' broom and frosty pod reaction has been described but is not very reliable. Efforts should be invested in determining whether the method could be refined to make it reliably detect resistance to these diseases.
Ceratocystis. The detached twig assay developed at INIAP (Carmen Suarez) is effective. Methods will be refined for use in Brazil (since strains there are more virulent, inoculum densities used in Ecuador, 75,000 cfus ml^{-1}, will be reduced).
Swollen shoot. Viruliferous mealybugs have been used in Ghana in disease screening trials. Disease progress is reasonably quick, but due to the existence of mild and severe strains of the pathogen, efforts would need to be made to ensure that all important variants of it were represented in trials designed to identify useful clones.

Vascular streak. Since this is caused by an obligate pathogen, work on this disease is difficult. Presently, artificial inoculations can only be done with basidiospores that are collected from affected cacao tissues. In seedling nurseries that are located in disease hot spots, symptoms develop within a month or two.

Quarantine/safe movement of germplasm

The IPGRI *Guidelines for the Safe Movement of Cacao Germplasm* (1989) needs to be revised and widely disseminated.

A kit developed under Jim Saunder's CRADA (USDA-ARS) by D^2 Biotechnology for detecting the *Phytophthora* spp., *C. perniciosa*, *M. roreri*, and *Cacao swollen shoot virus* needs to be widely tested for reliability against diverse strains of the different pathogens (It is currently being tested at the University of Reading). Efforts should also be made to add *O. theobromae* detection to the kit.

Host range

At least two groups have indicated that the C-biotype of *C. perniciosa* may be a pathogen of solanaceous plants as well as cacao. Although specific information is not available, it appears that inoculum density may play a critical role; i.e., symptoms develop on solanaceous plants only when heavy doses of basidiospores are involved. Work needs to be done to determine whether work with the C-biotype presents undue risk in areas where solanaceous crops are grown.

Although it is agreed that *Cacao swollen shoot virus* is not endemic in the Americas, there is some debate as to where the pathogen originated. Reports from the 1950s indicated that *Kola* sp. (Sterculiaceae) was the original host in West Africa, but the world authority on the badnaviruses (Ben Lockhart, University of Minnesota) indicates that the virus may in fact have originated in Asia. It has a wide host range, including species in the Araliaceae, Bombacaeae and Sterculiaceae. Since the original reports on *Kola* sp. demonstrated a very low rate of infection on this host, work to clarify the virus' origin and complete host range is warranted. This impacts not only the management of this disease, but also its movement during the international exchange of germplasm.

Among the black pod pathogens, only *P. megakarya* is host specific; *P. capsici, P. citrophthora*, and *P. palmivora* each have wide host ranges. Work need to be done determine the host ranges of strains of the latter species in order to gauge whether significant risk is associated with their use in biocontrol and other studies. Currently, John Bowers (USDA-ARS Beltsville) is investigating genetic relationships among strains of *P. capsici*, but plans work on the host range of it and cacao strains of *P. citrophthora* and *P. palmivora* on other crop species.

Pathogen diversity

Work on the *Phytophthora spp.* and *C. perniciosa* is warranted. Research on *Cacao swollen shoot virus* has been published and on *M. roreri* is being completed (Wilbert Phillips, CATIE).

Previous research indicated that genetically and pathogenically variable populations of *C. perniciosa* were present in tropical America. Since their presence in different production areas will impact the performance of new clones it is imperative that they be better understood and that their geographical distributions be known. Work with

a hierarchically sampled collection of the pathogen is underway in Brazil at CEPLAC (Itabuna, Karina Gramacho) using SSR variation, and work with geographically diverse isolates is in progress at UF (Homestead, Randy Ploetz) using somatic incompatibility and, in the future, SSRs. The UF lab also plans to characterize pathogenic variation in the pathogen using diverse clones of cacao. Collections of the pathogen from areas in which it is thought to have co-evolved with cacao (e.g., the Oriente region in Peru) are needed.

Biocontrol

There is considerable research in this area by MARS (Prakash Hebbar), USDA-ARS (John Bowers et al.), CATIE (Ulrike Krauss), CEPLAC (Jose Bissera) and others. Good initial progress has been made in this area, and there is good collaboration among the various groups. There was a clear consensus that these cooperative efforts should continue. Note was made of a Level 3 containment facility that would open soon in Beltsville (USDA-ARS) which could be used to evaluate exotic biocontrol agents. Also, there may be a mid-year meeting among the players in this area this year, possibly in Beltsville.

Cultural and chemical control

The discussion centered on the efficacy and difficulty of using cultural practices (mainly the removal of symptomatic tissues from plantings) to manage the various diseases. Although published work (Ulrike Krauss) documents the effectiveness of phytosanitation for witches' broom control, it is labor intensive and difficult to impose in large trees (see case made below for dwarfing rootstocks).

Diverse chemicals have been tested against the various diseases. It was recognized that the economics and environmental impacts of the various compounds needed to be recognized whenever chemical disease control was considered. Many of the newer fungicides (e.g., the strobilurins) are effective against many diseases and environmentally benign, but are also quite expensive. Others that are effective against some of the diseases and relatively inexpensive (e.g., the copper-containing products) have been associated with negative impacts on soil microflora. A summary of results for chemical products against different diseases in different locations is needed.

EPIDEMIOLOGY

Good work has already been conducted on witches' broom and black pod (Bob Schmidt et al.). Additional work on frosty pod and swollen shoot is needed to better understand these important diseases. Very little is known about the epidemiology of frosty pod, and based on current knowledge of the badnaviruses, the host range and the origin(s) of *Cacao swollen shoot virus* should be re-investigated. In addition, proceedings of an epidemiology meeting that took place in Colombia should be translated to English since it contains much good information.

Rootstocks

The impact of rootstocks on cacao productivity should be investigated. Although Rob Lockwood detailed previous failures to identify beneficial rootstocks during the meeting, it appears that no work has been done to identify disease resistant rootstocks. Since

several of the important pathogens of cacao are soilborne or have significant portions of their life cycles on the host root system (especially the *Phytophthora* spp.), it is clear that resistant rootstocks have the potential to ameliorate some of these diseases. Research on this objective should be initiated. Likewise, great benefit was envisaged for dwarfing rootstocks. If they could be developed and used to produce smaller (i.e., "pedestrian friendly") trees, cultural management of the important foliar diseases would be much easier.

Action points

1. There is a critical need to develop a reliable rapid screening technique for determining resistance to frosty pod. The seedling assay of Harry Evans (1978) should be refined and examined for reproducibility.
2. A kit developed under Jim Saunder's CRADA by D^2 Biotechnology for detecting the *Phytophthora* spp., *C. perniciosa*, *M. roreri*, and *Cacao swollen shoot virus* needs to be widely tested for reliability against diverse strains of the different pathogens. *O. theobromae* should be added to the list of pathogens that are detected by the kit.
3. The IPGRI *Guidelines for the Safe Movement of Cacao Germplasm* (1989) needs to be revised and widely disseminated.
4. Work needs to be done in order to determine the risk that is posed by work with the C-biotype in areas where solanaceous crops are grown.
5. Comprehensive information on genetic and pathogenic variation in the important pathogens, particularly *C. perniciosa*, *Cacao swollen shoot virus*, and cacao strains of *P. capsici, P. citrophthora* and *P. palmivora*, is needed.
6. A pathology working group that was established at this meeting should be expanded to include all of the significant players in this area. Periodic meetings of the group should be scheduled during which members would discuss their research and objectives for future work.
7. Work to identify disease-resistant and dwarfing rootstocks should be initiated.
8. Efforts should be invested to determine whether the sap test for witches' broom and frosty pod reaction could be refined to make it more reliable for predicting field resistance.
9. Results for chemical products against different diseases in different locations should be summarized.
10. Work should be initiated on the epidemiology of frosty pod and swollen shoot.
11. Proceedings of an epidemiology meeting that was held in Colombia should be translated to English.

The chair would like to thank Cecile Olano and Prakash Hebbar for taking notes during the session and interacting during the preparation of this report. He also apologizes for any mistakes or omissions there may be above. Please forward these oversights to him for future reference at: rcp@mail.ufl.gnv.edu

VISIT OF TREC-UF LAB

On the afternoon of 23 January, the following scientists visited TREC-Homestead to discuss disease research on cacao and the facilities at TREC:

Brian Bailey (USDA-ARS)
Julie Flood (CABI)
Karina Gramacho (CEPLAC)
Prakash Hebbar (MARS)
Lizz Johnson (CRU)
Uilson Lopes (CEPLAC)
Bob Lumsden (ACRI)
Wilbert Phillips (CATIE)
Bob Schmidt (UF)
Carmen Suarez (INIAP)

The chair thanks the above group for providing significant information and feedback on ongoing and planned experiments in his lab. A final thanks goes to Wilber Q. for his help as a chauffeur.

Administrative Coordination

Administrative/Cooperative Linkages

- Reading Database
  - Beneficiaries – Producer Countries
  - BCCCA funds and administers
  - Too many errors in database
  - More user friendly?
  - Possibly have INGENIC perform data management
  - New funds needed for data maintenance?
  - Requires USDA support

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Scientific Steering Group?

- If so, should look like Arabidopsis.
- Formulate as subcommittee of INGENIC

Global Cocoa Programme

- Two (2) years under aegis of ICCO
- CABI and CIRAD have taken lead
- Successes to date: Montpellier in September, 2000. Agreement on 15 markers
- Only working collaborative body –should be formalized
- Relationship with ICCO on hold until new leadership apparent
- Funding base ($35,000) needed to support conferencing

Intellectual Property

- Results of International collaborative activity should be in public domain

Report on the joint USDA/INGENIC discussion meeting on the creation of an "International Working Group to Study the *Theobroma cacao* Genome"

First, an introduction was given by Bertus Eskes on the activities of the International Group for the Genetic Improvement of Cocoa (INGENIC) over the first seven years of its existence. The INGENIC mailing list contains now addresses of 350 interested persons. Three workshops were organized so far and six Newsletters were edited. During the last workshop, held in Malaysia in October 2000, a proposal to set up a consortium for cocoa genomic studies was launched by Mark Guiltinan. The INGENIC board endorses this important initiative and has received positive reactions from INGENIC e-mail correspondents informed on the intention to create a group dealing with genomic studies. INGENIC is grateful to USDA to have provided the possibility for discussions on this matter during the present Cocoa Research Meeting.

As an example, the Banana Genome Consortium was briefly depicted. Two meetings were organized to set up the banana consortium that contains the following objectives:

- unite forces to obtain common non-competitive research objectives of global interest;
- results are in the public domain;
- main activities relate to the development of tools and use of genome knowledge from other species;
- facilitation is provided through a coordination unit at INIBAP, Montpellier;
- although participants have initiated their activities already, additional fund raising is proposed (EU and Brazilian sources).

A document will soon be published on the banana initiative which can be requested by any interested person from Claudine Picq at INIBAP (c.picq@cgiar.org).

The discussions continued with presentations by Claire Lanaud and Julio Cascardo on how genomic tools work and what can be expected from genome studies in cocoa.

After the break, an open discussion was initiated on the creation of a group on cocoa genome studies. It was recognized that the fruitful discussions that had been carried out the day earlier already identified a common research goal related to EST libraries and bioinformatics. The present discussion session was aimed at following up on these earlier discussions within a larger group and aiming at broader objectives.

The following name was proposed: 'International Working Group on Cocoa Genome Studies'. Proposed as main objective was: "to identify and share molecular genetic information to improve cocoa varieties". Results should be in the public domain. Main activities were identified as: creation of EST libraries, micro-array studies, increase of SSR markers, BAC mapping, genotype identification studies, creation and management of segregating populations and database management.

The institutions involved in the group would include initially: CEPLAC, CIRAD, MARS Inc., PennState University, USDA, UESC and possibly the new CFC project (for the creation and management of segregating populations). Other possibly interested institutions (Reading University, Unicamp, CNRA) will be contacted by INGENIC.

For the organization of the group, the first step discussed was the creation of a 'scientific committee', made up by one representative from each participating institution. This committee will take initiatives to further develop the activities of the group. The following persons accepted during the meeting to be part of this committee: Julio Cascardo, Mark Guiltinan, Martin Gilmour, Philippe Petithuguenin, Ray Schnell and Uilson Lopes. It was suggested that the group could act as a sub-group of INGENIC. The INGENIC chairman will discuss this further with the INGENIC committee and inform the group members accordingly. A chairmanship for the group needs to be identified by direct contacts between the group members. Possibly, the chairmanship could be done on a rotative basis.

One of the first activities of the group would be to prepare a strategy document aimed at a larger public that would explain the main objectives and working procedures of the group. In a second stage, the need for additional funding of activities will be analyzed. The group would tentatively meet within one year to discuss further developments and activities.

It is recognized that the collaborative group on EST libraries, created earlier during the week and chaired by David Kuhn, is specifically looking into collaboration on EST activities and bioinformatics (see report on the discussion group). The relationship between this group and the broader aiming 'International Working Group on Cocoa Genome Studies' will need to be clarified.

The INGENIC chairman will inform e-mail respondents on the outcome of this meeting. The USDA will prepare a note for the INGENIC Newsletter on the creation of the collaborative groups on cocoa genomic studies.

Bertus Eskes
INGENIC chairman

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